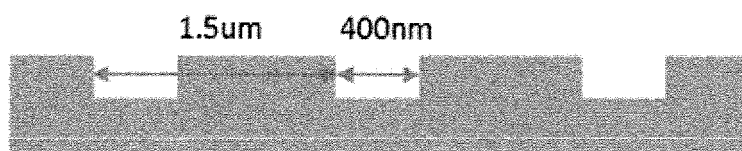
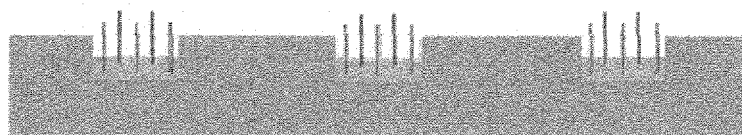
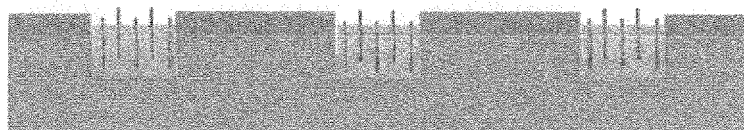


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1. Patterned Nextseq substrate



2. PAZAM polish, graft P5/P7

3. 2nd layer of PAZAM or SFA

4. Graft with phosphate terminated P5/P7

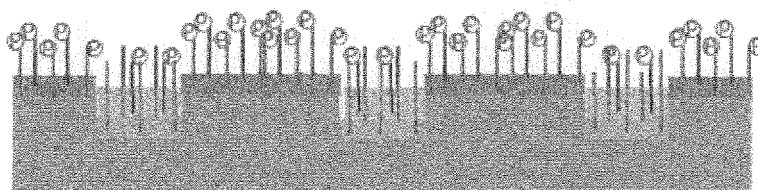
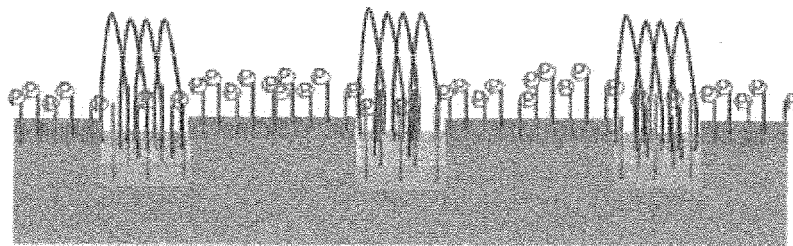


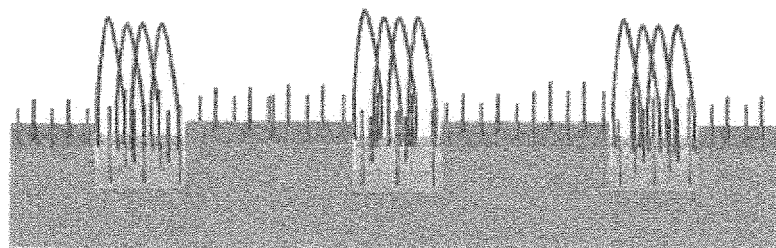
FIG. 1

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5. KEA inside nanowell



6. T4-Kinase



7. Bridge amplification or KEA to enlarge the cluster

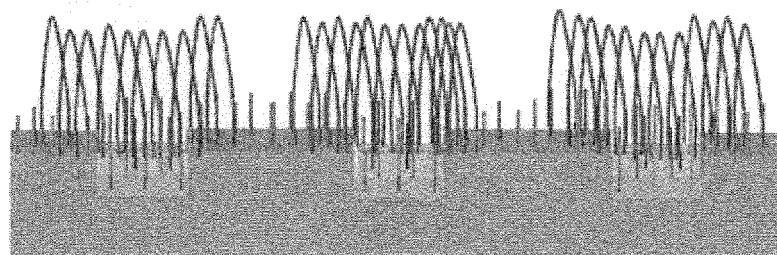
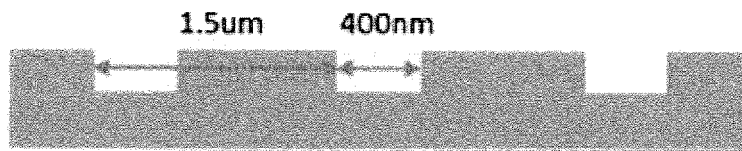


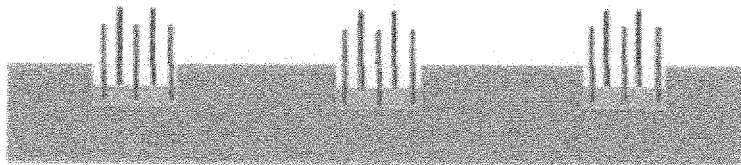
FIG. 1

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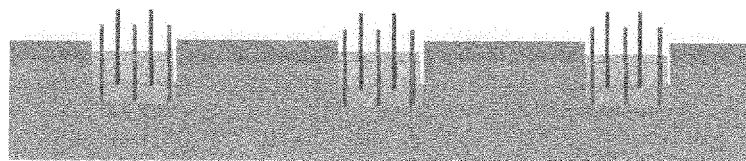
1. Patterned Nextseq substrate



2. PAZAM polish, graft P5-SBS3/P7-SBS8 primer



3. 2nd layer of PAZAM or SFA



4. Graft with regular P5/P7

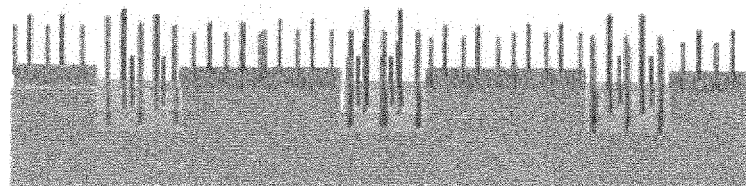


FIG. 2

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6. KEA

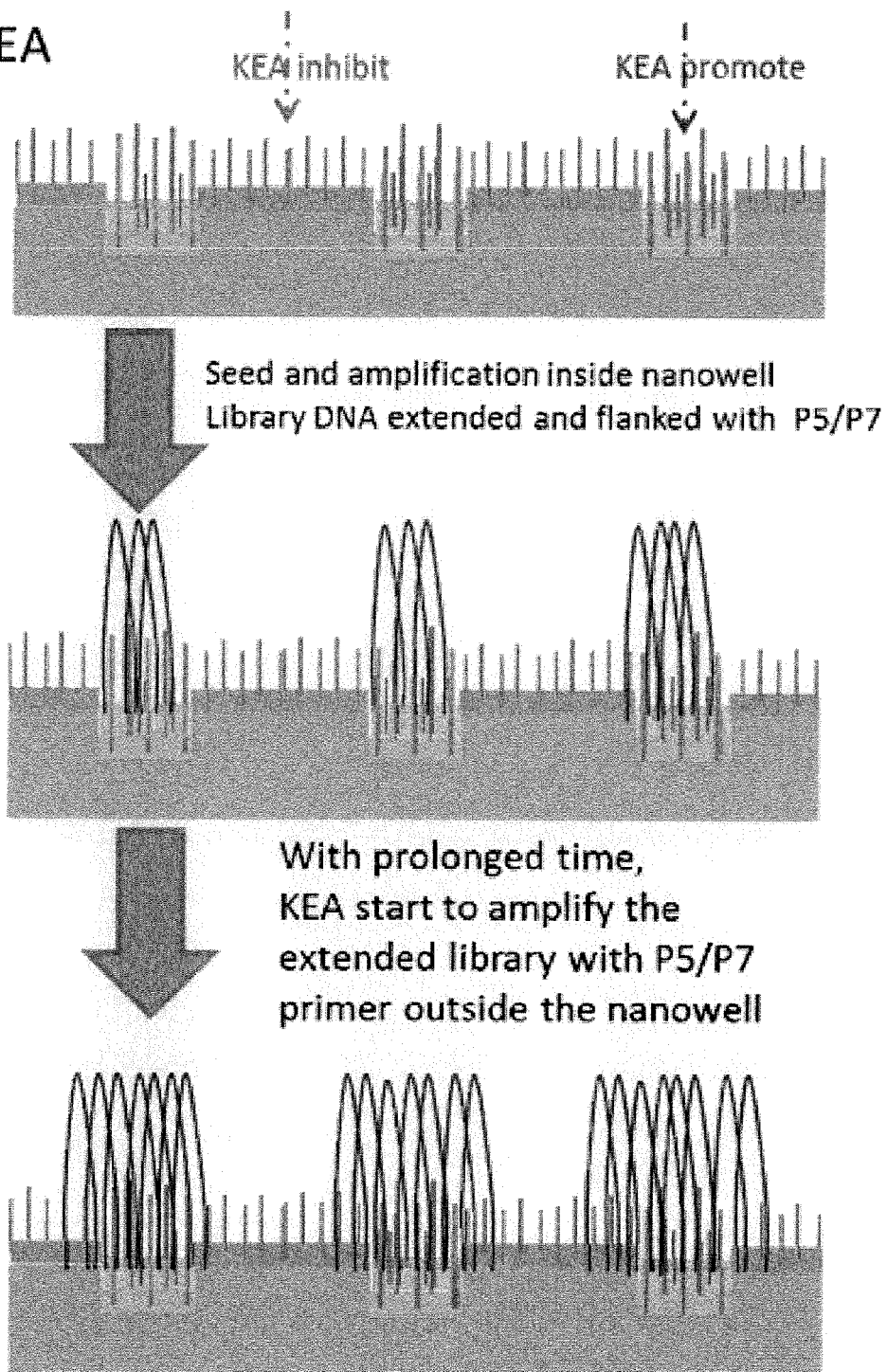
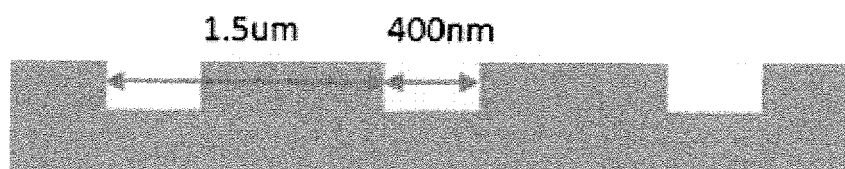


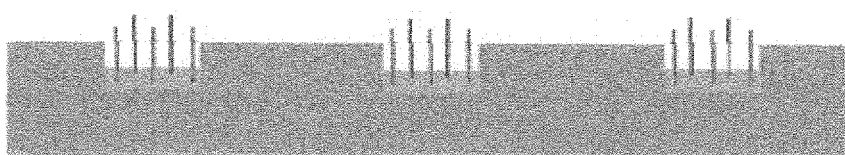
FIG. 2

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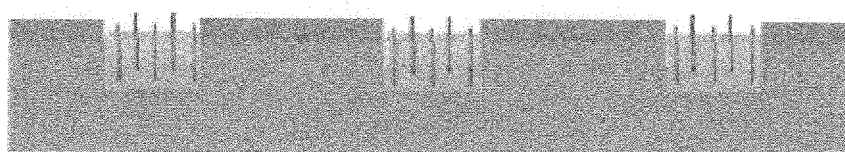
1. Patterned Nextseq substrate



2. PAZAM polish, graft P5/P7



3. 2nd layer of PAZAM or SFA



4. KEA

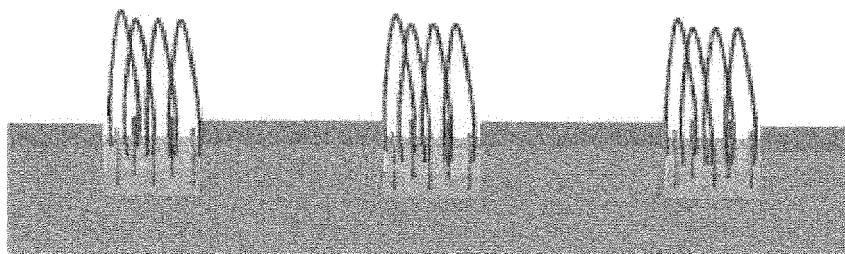
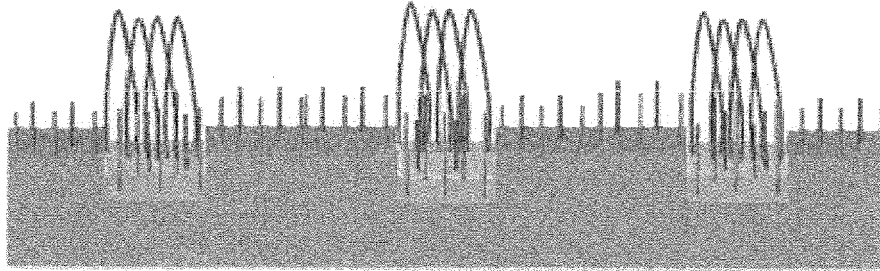


FIG. 3

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5. Second graft with P5/P7



6. Bridge amplification or KEA to enlarge the cluster

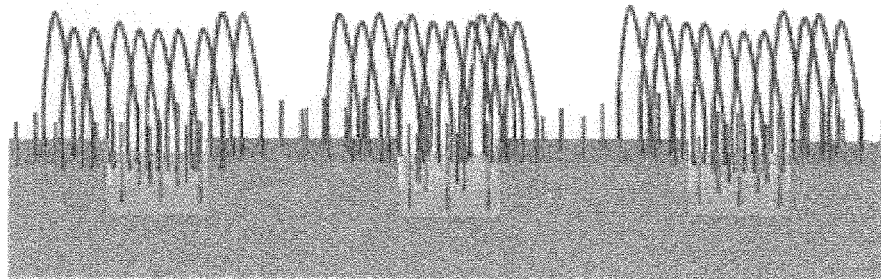
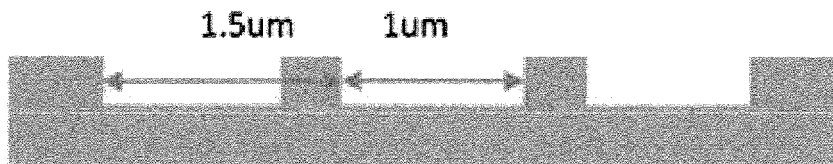


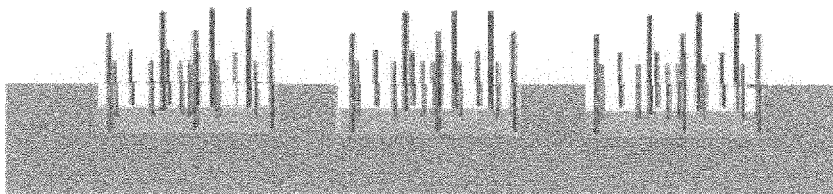
FIG. 3

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1. Patterned Nextseq substrate



2. PAZAM polish, graft P5/P7 and P5-SBS3/P7-SBS8



3. KEA with library flanked with SBS3/SBS8

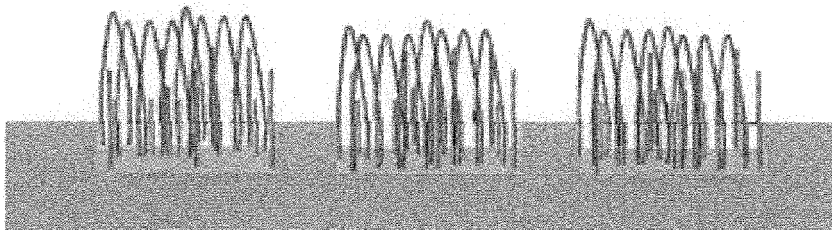


FIG. 4

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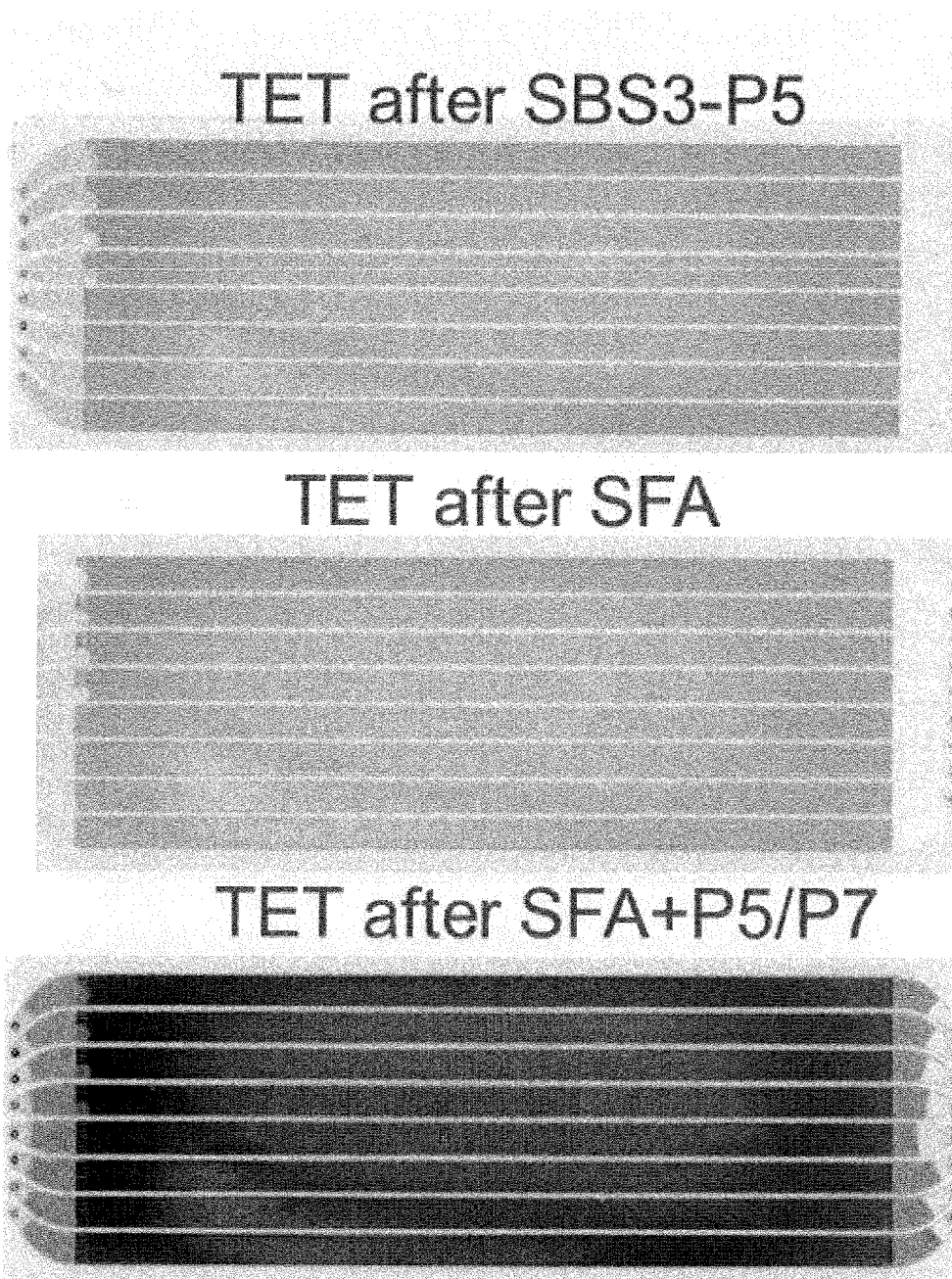


FIG. 5

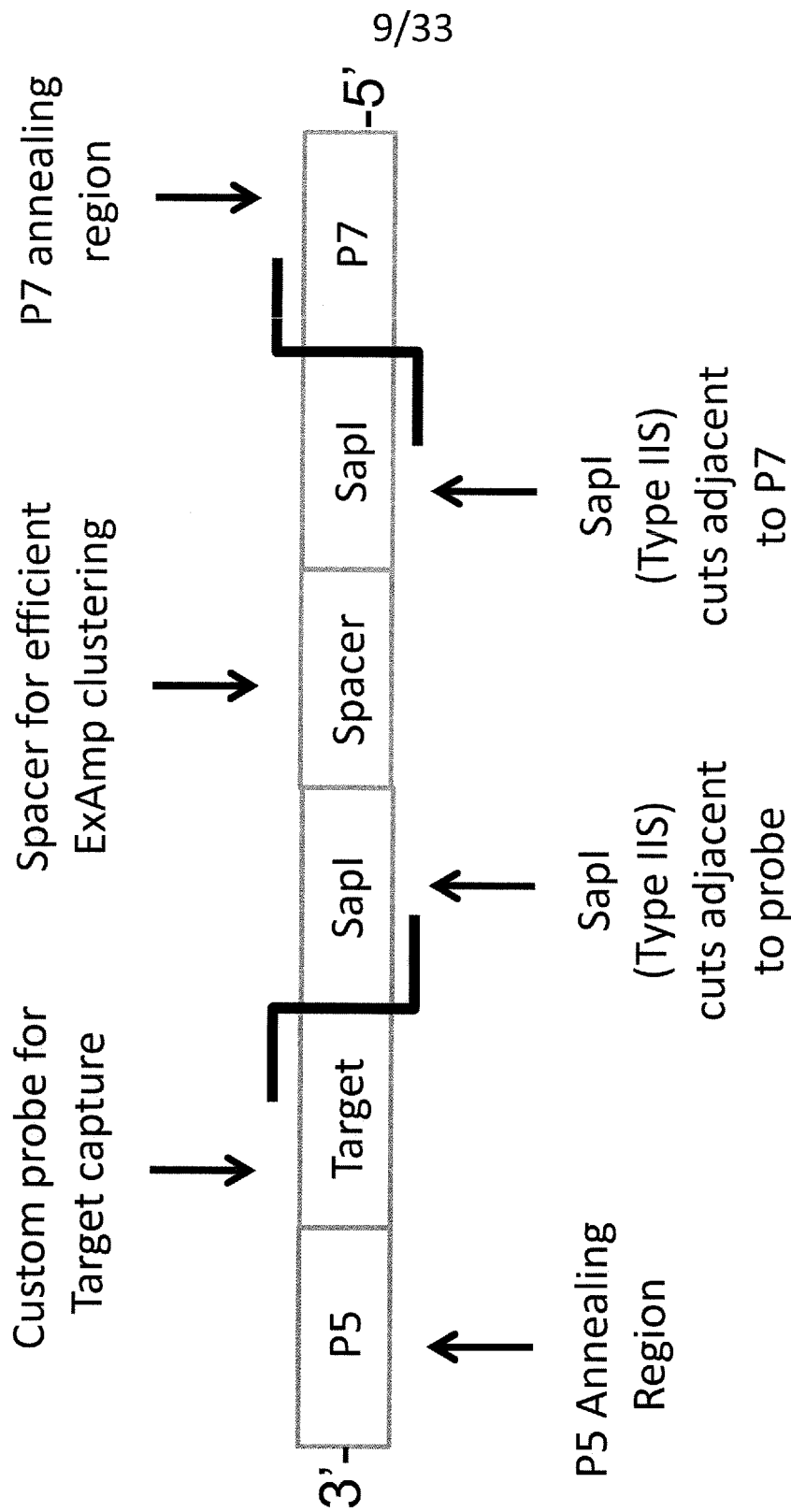


FIG. 6A

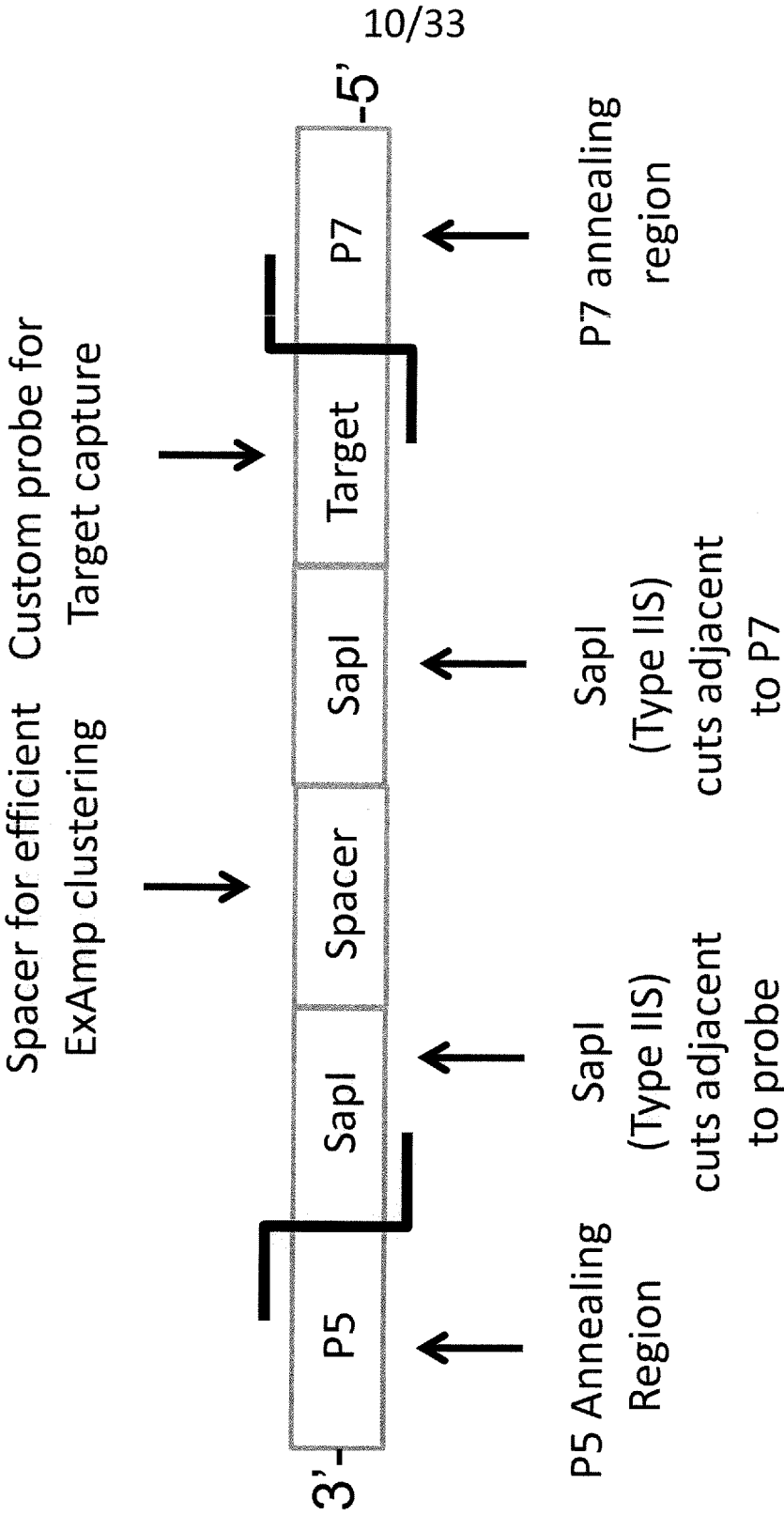


FIG. 6B

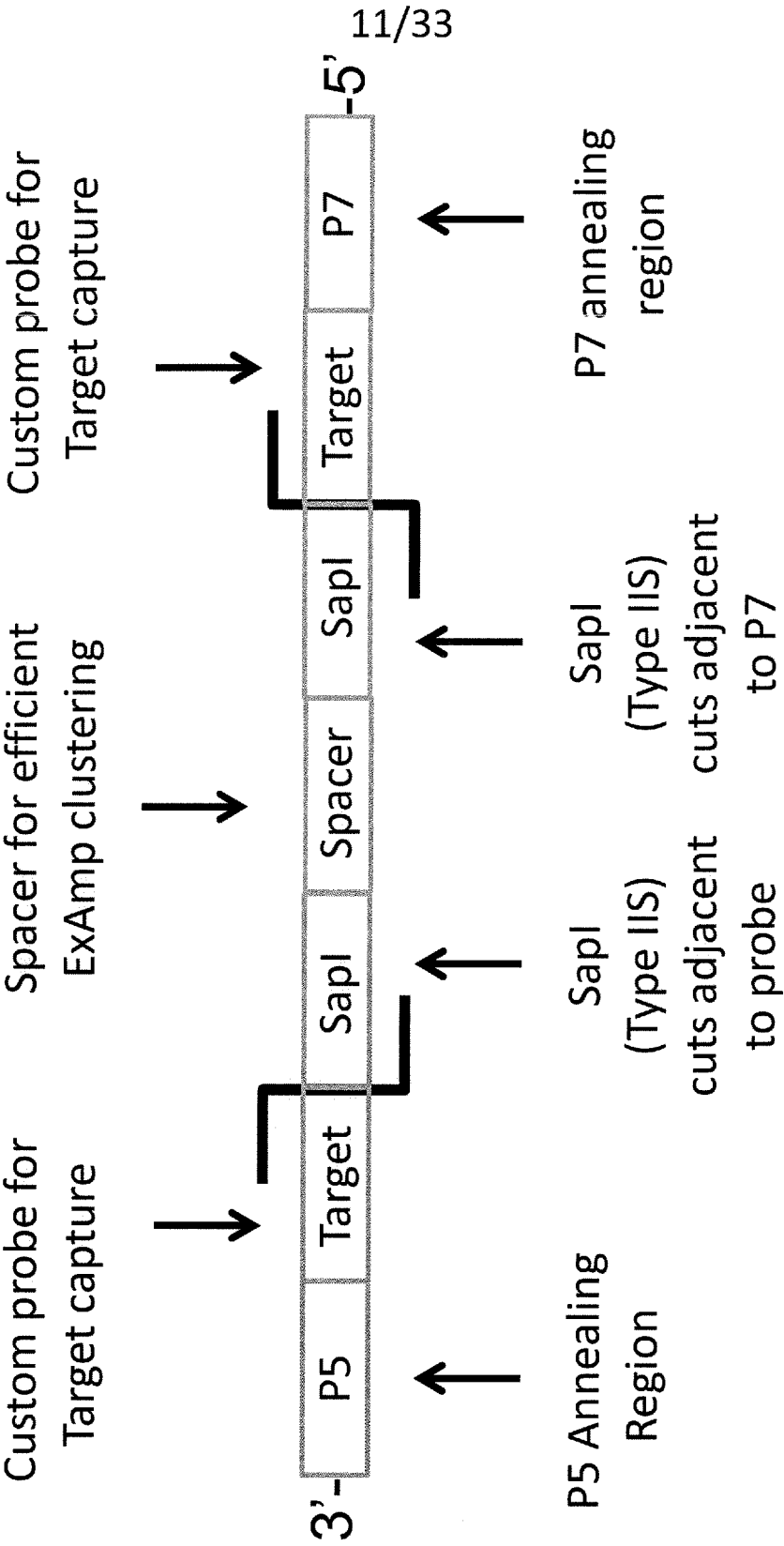


FIG. 6C

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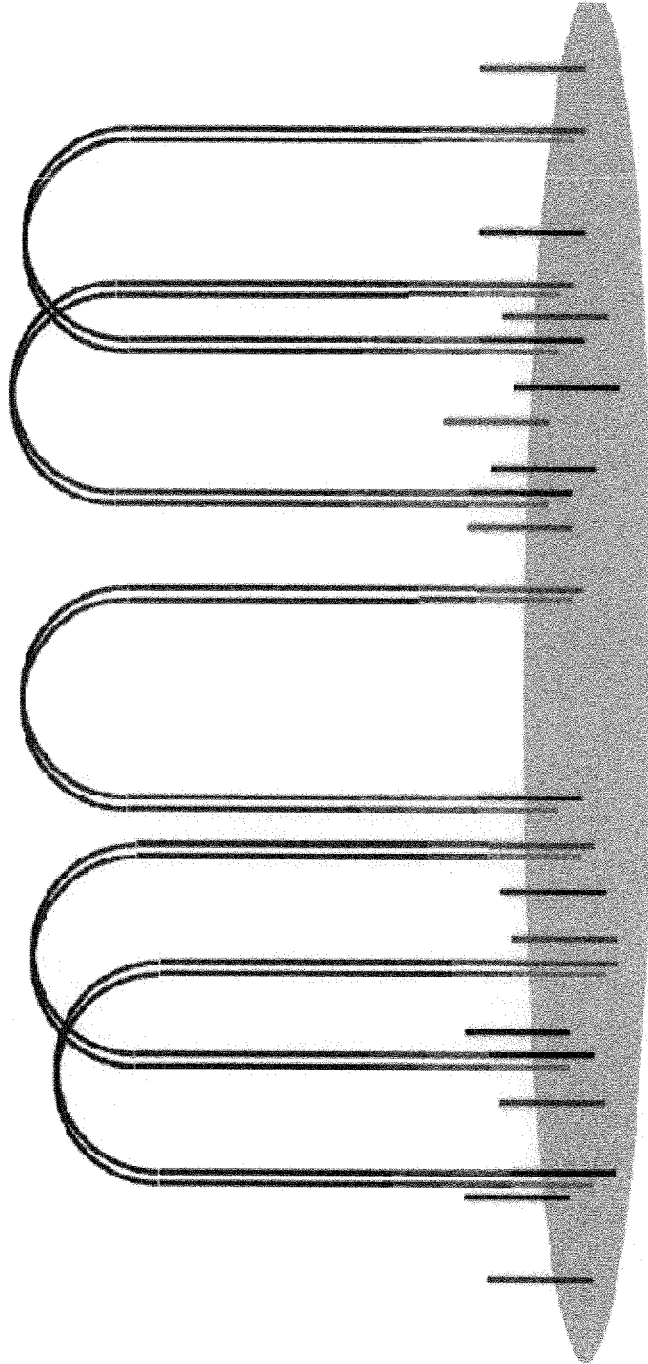


FIG. 7A

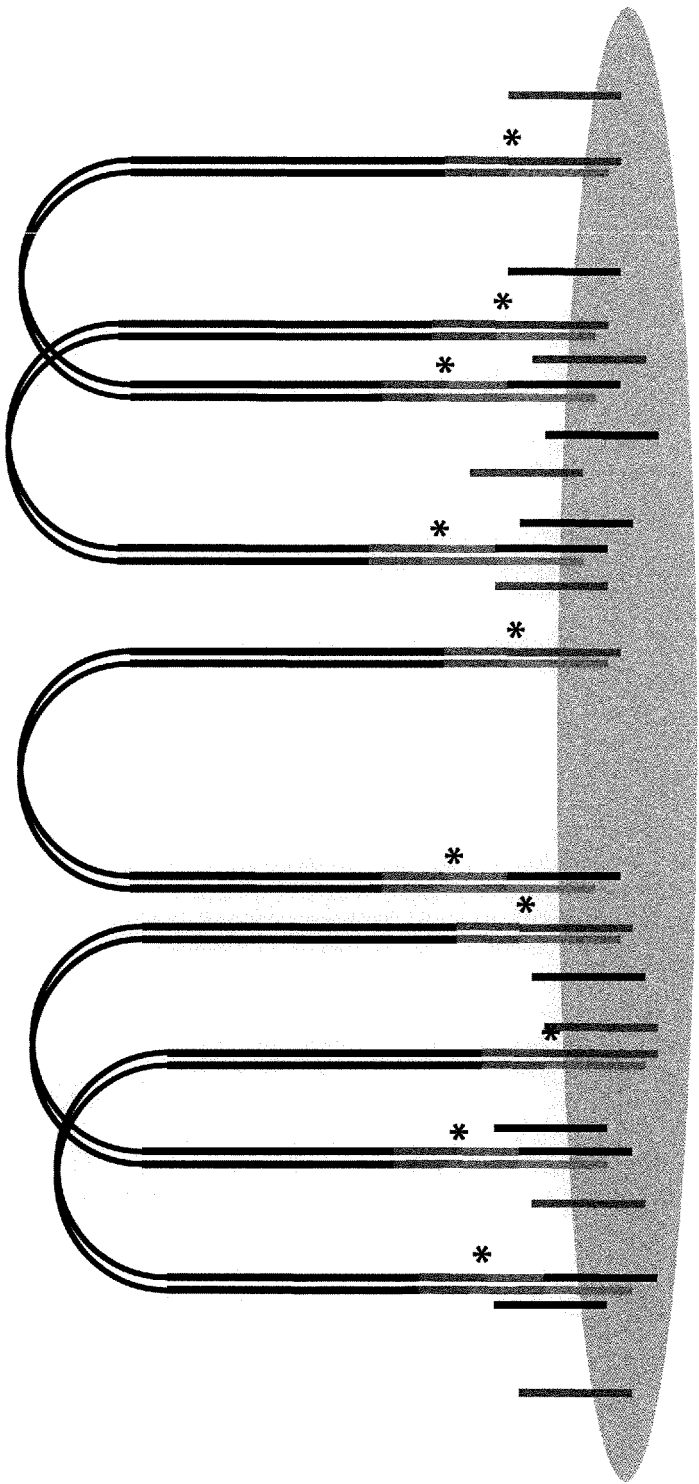


FIG. 7B

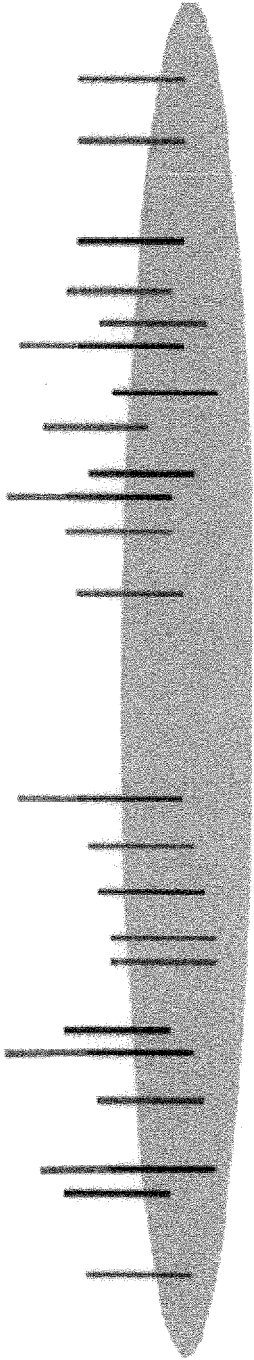


FIG. 7C

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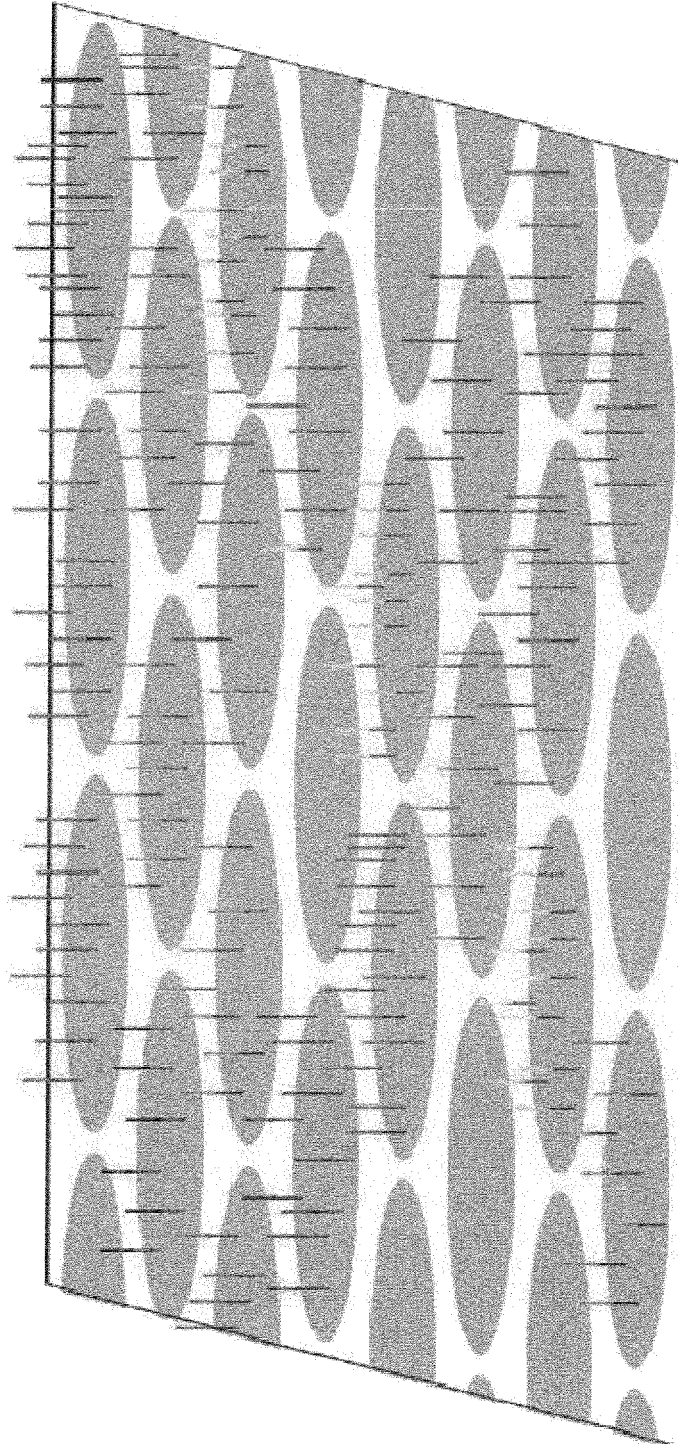
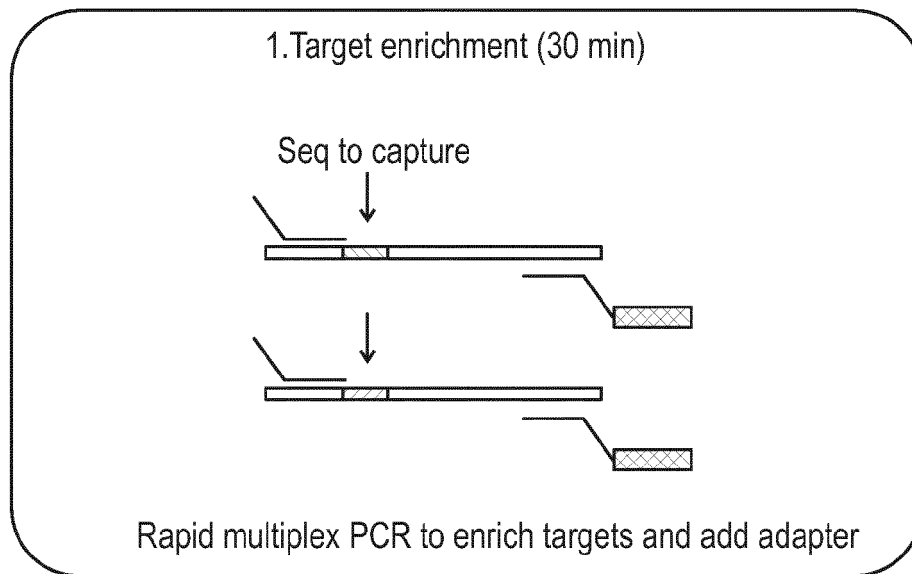


FIG. 7D

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A



B

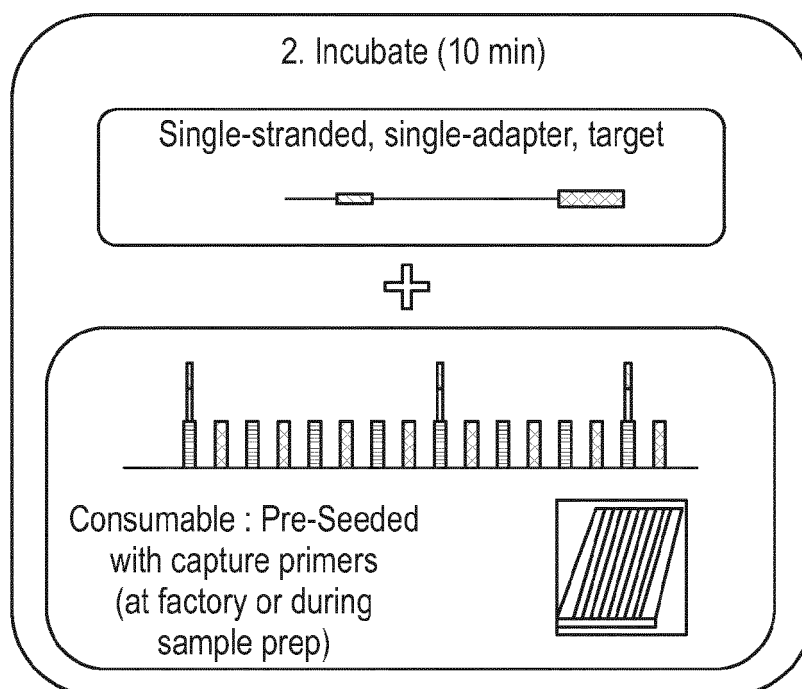


Fig. 8A and B

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C

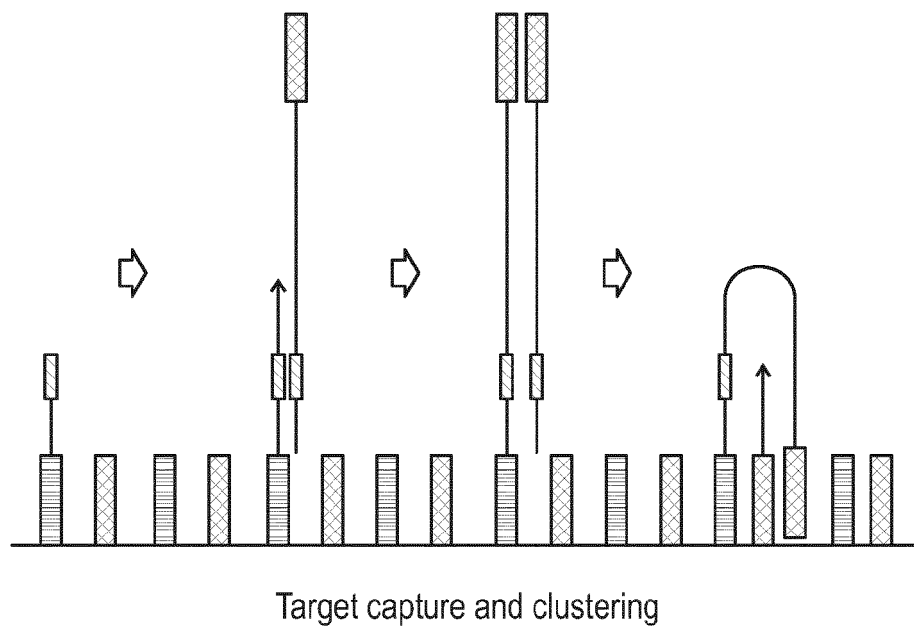


Fig. 8C

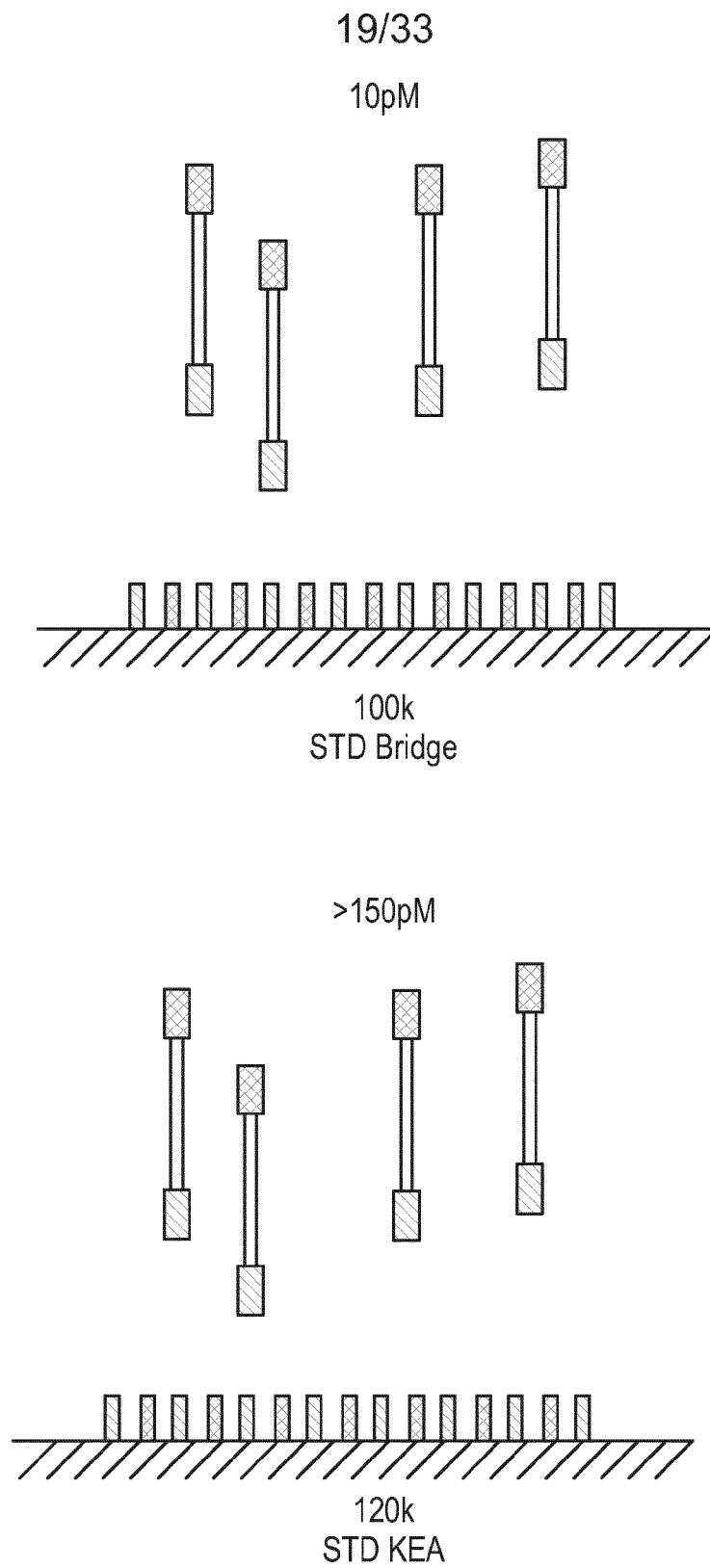


Fig. 9A and B

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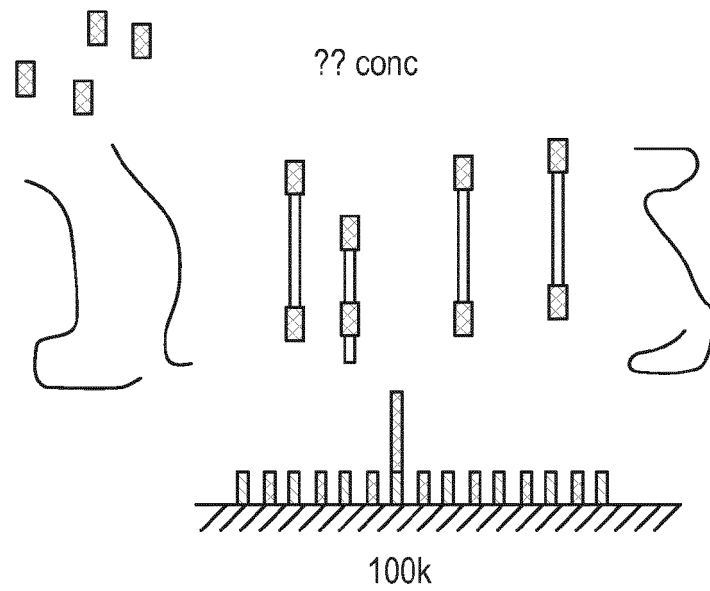


Fig. 9C

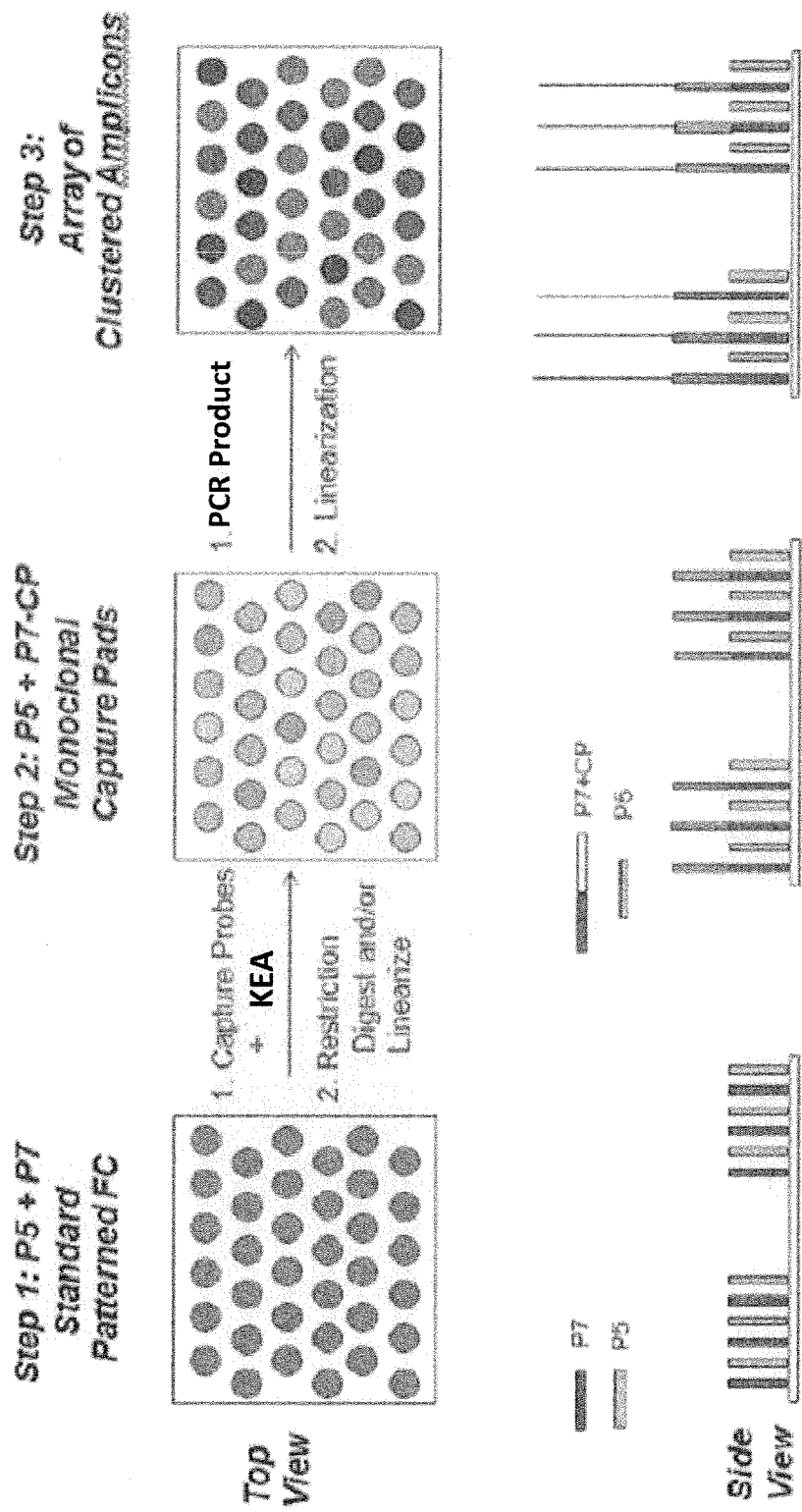


FIG. 10

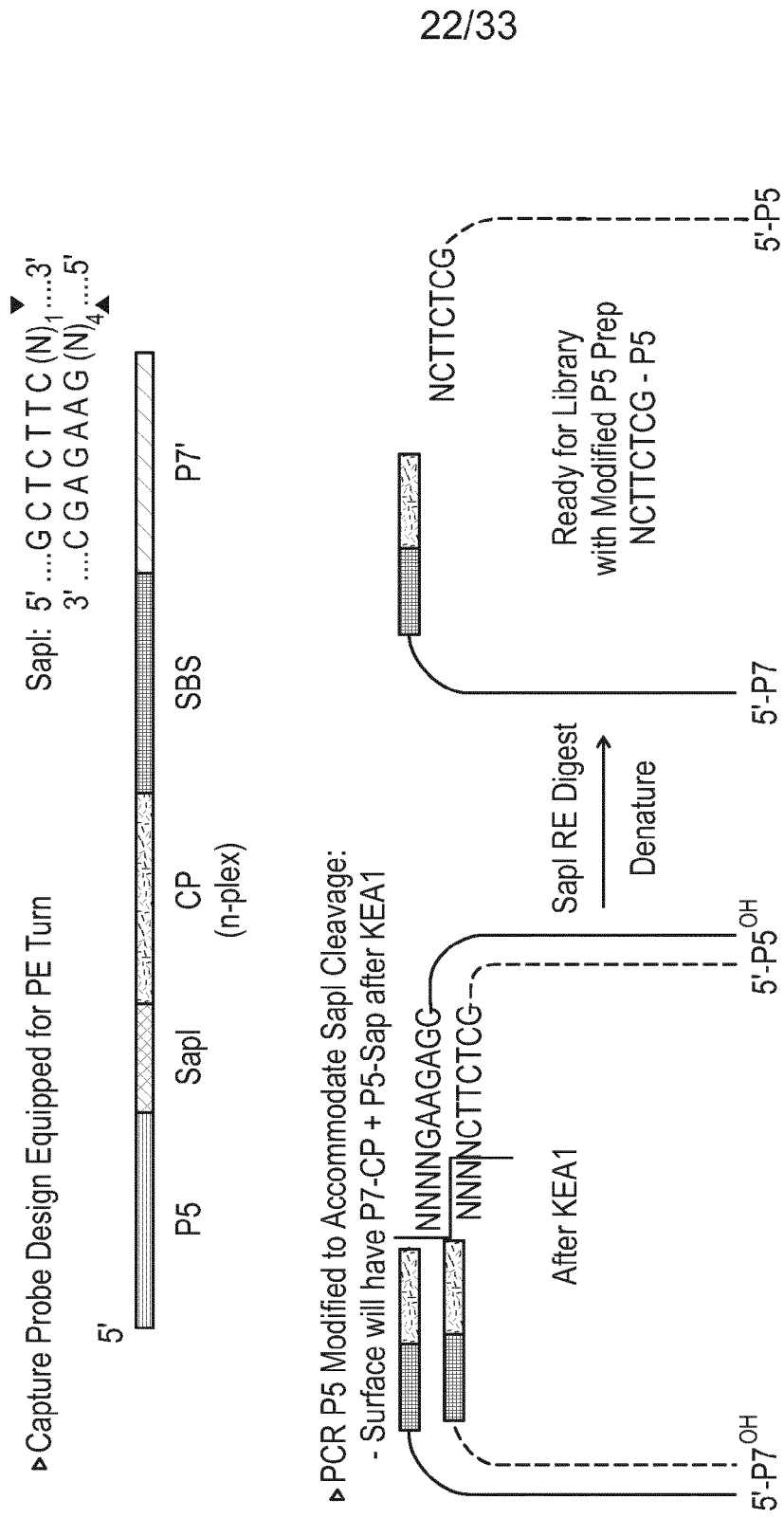


Fig. 11

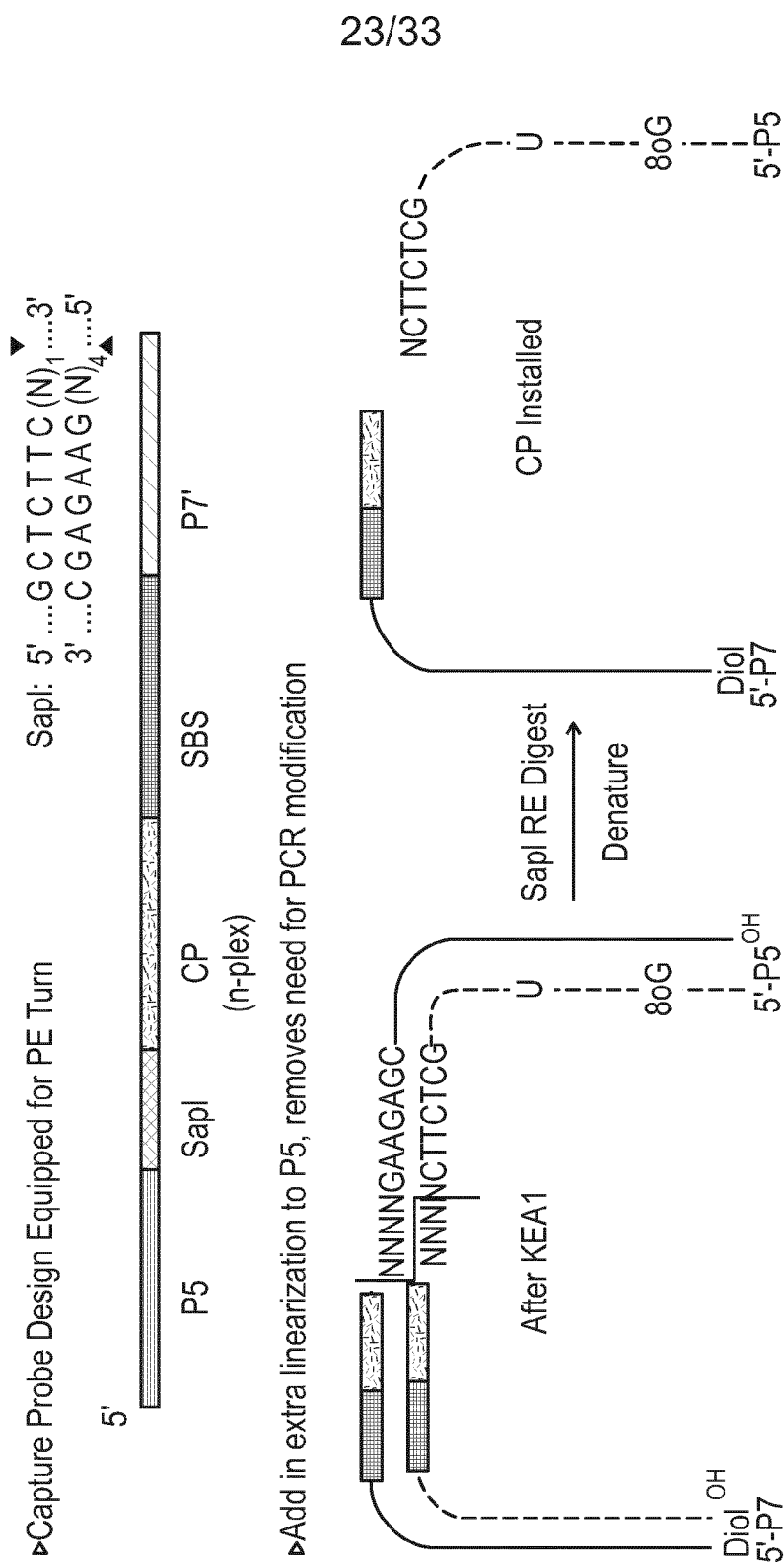


Fig. 12A

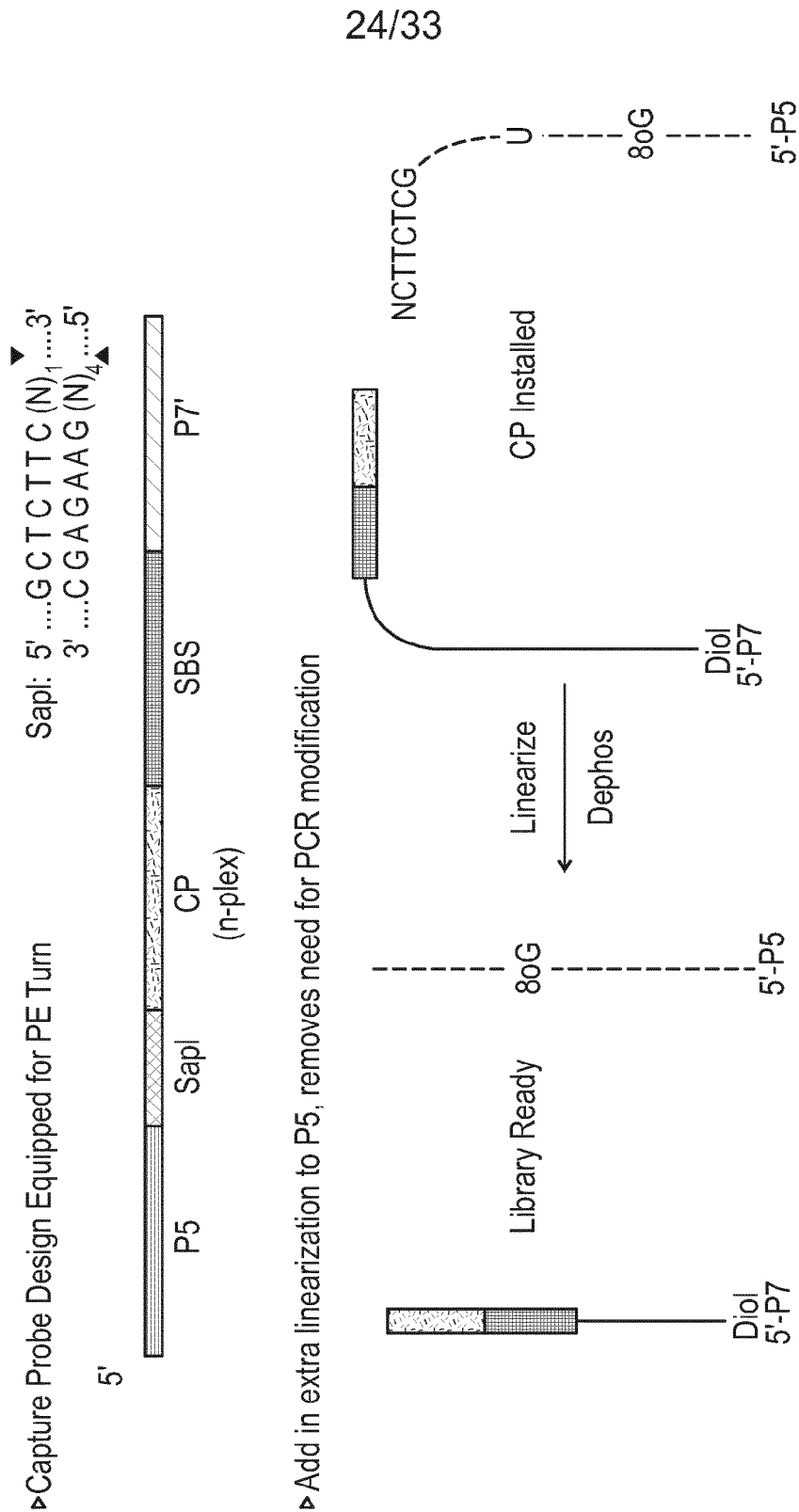


Fig. 12B

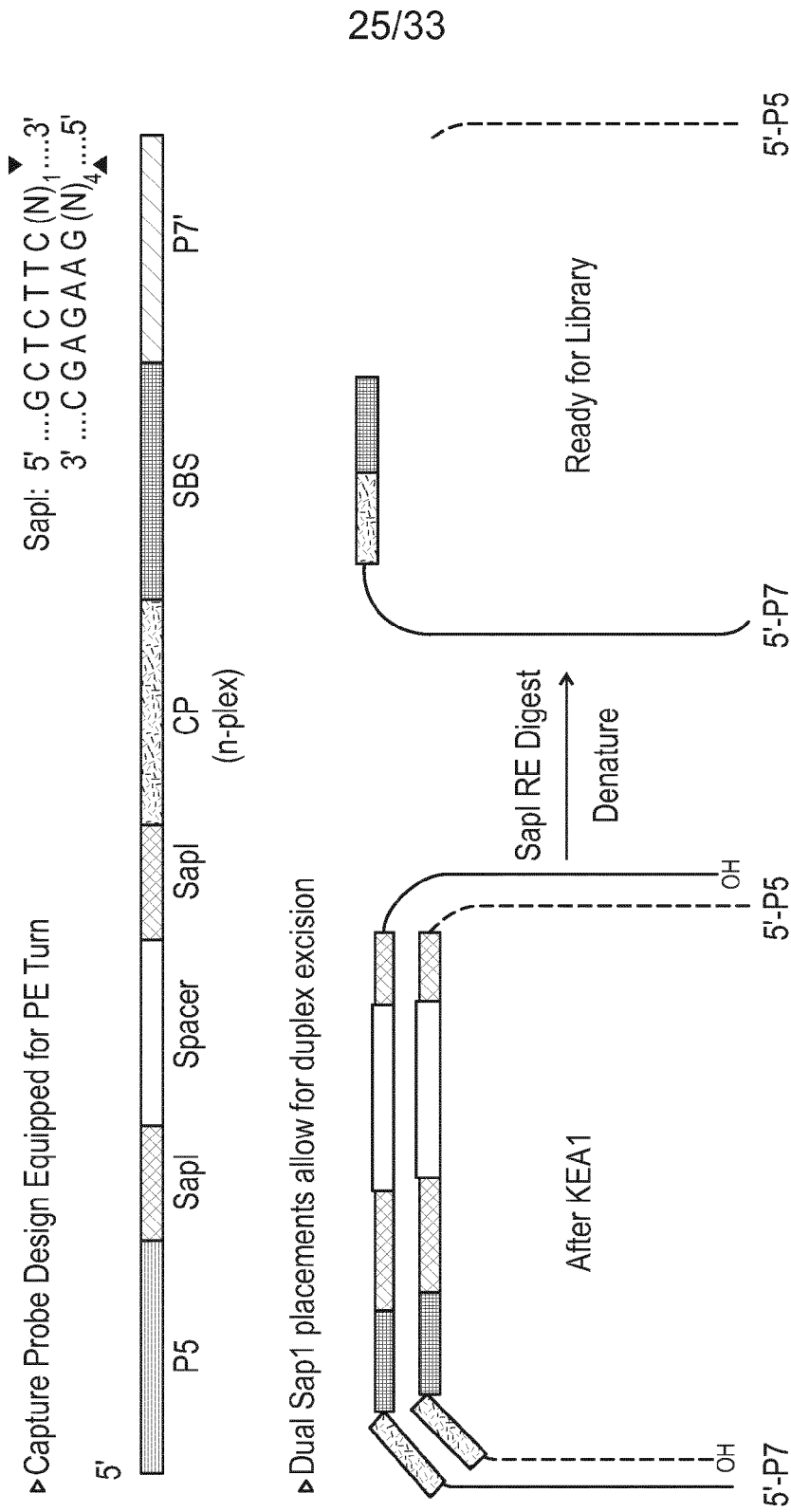
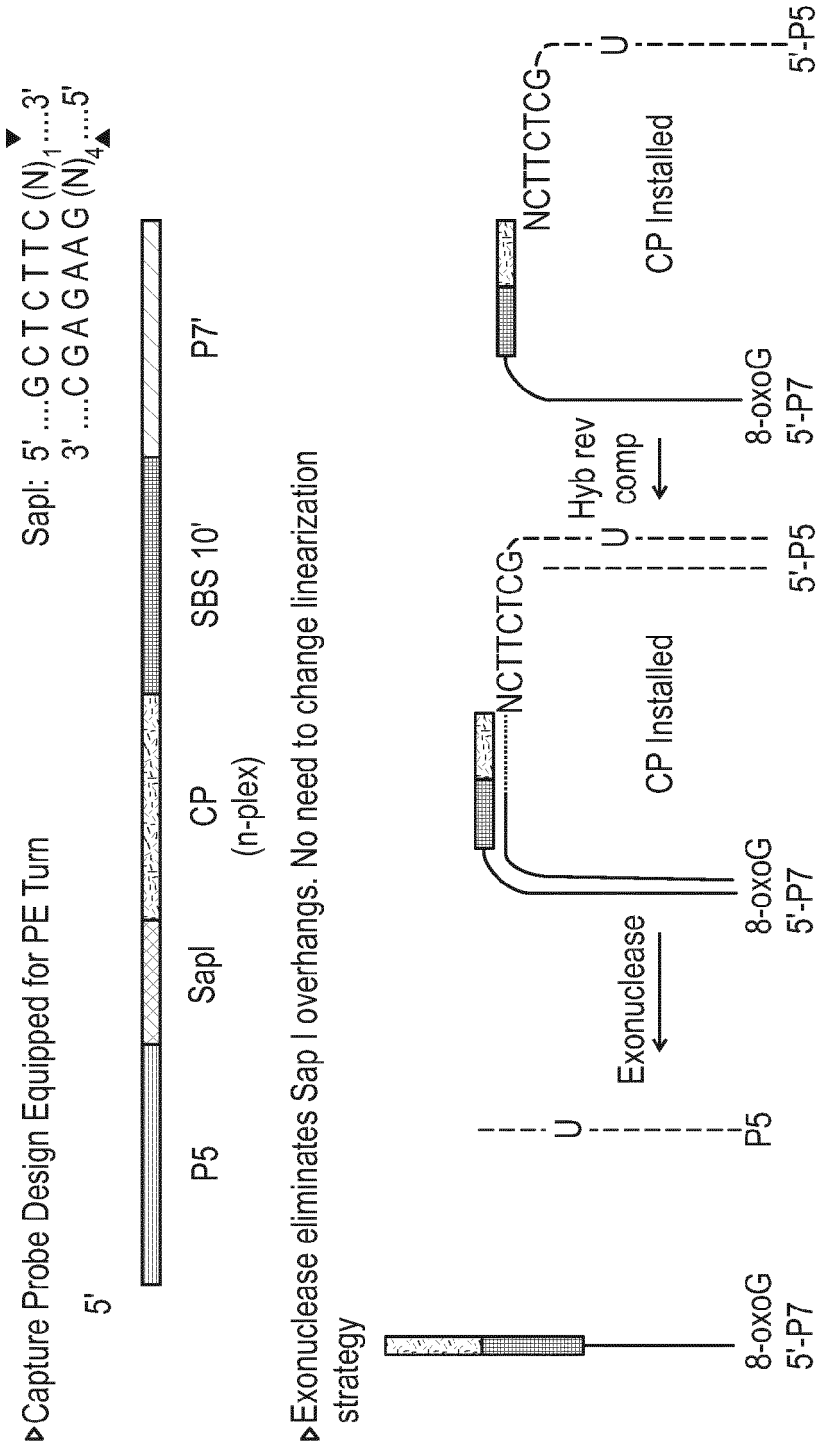


Fig. 13



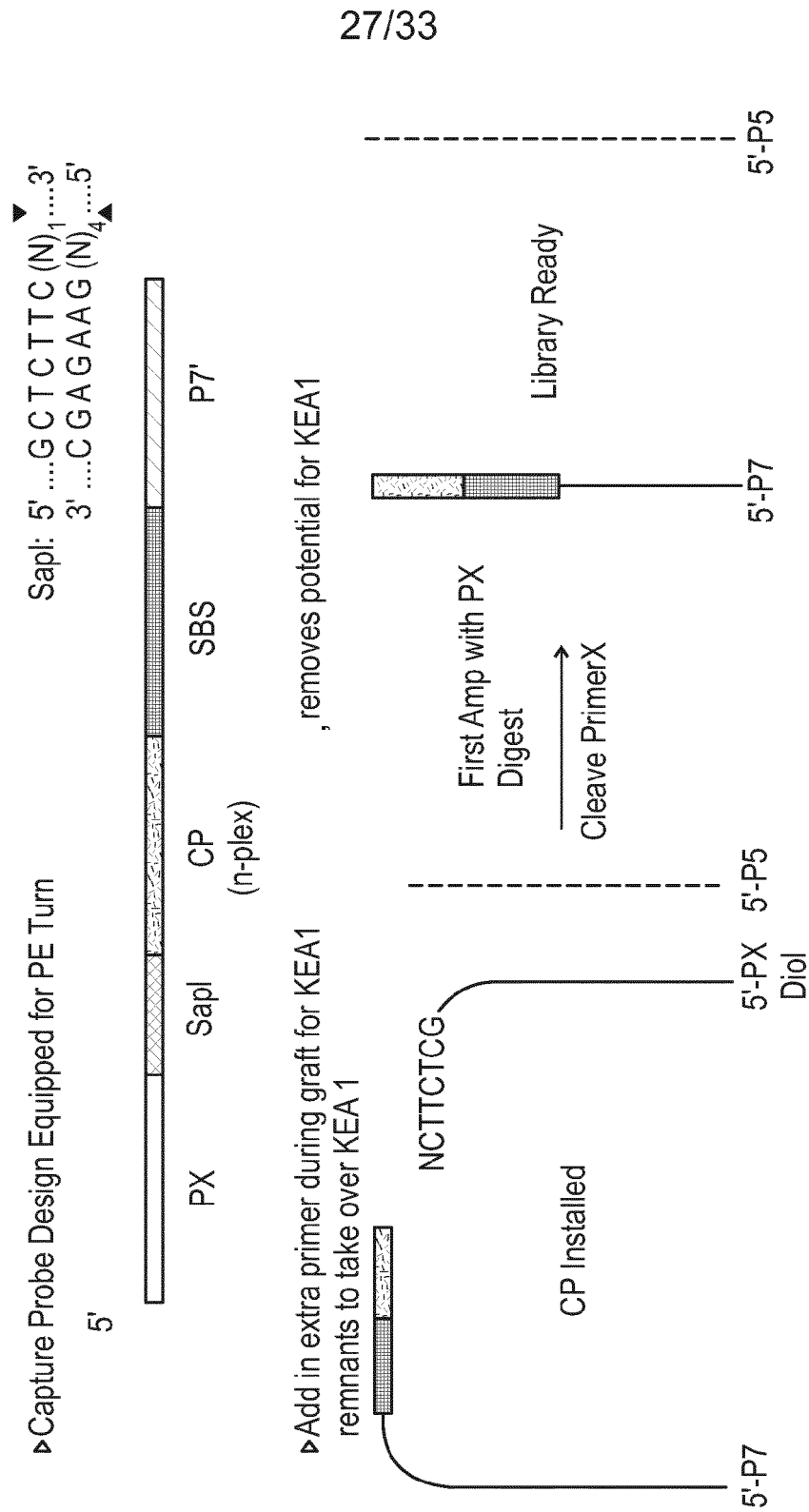


Fig. 15

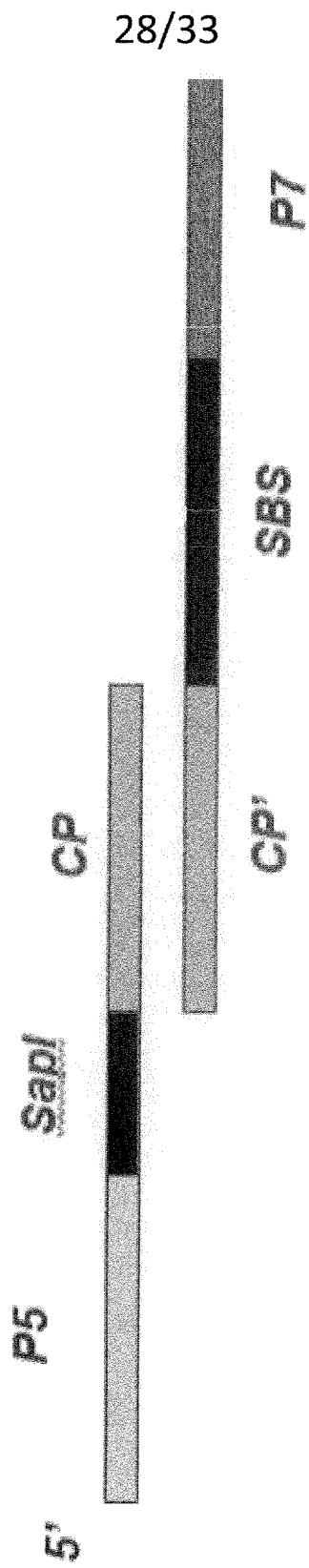


FIG. 16

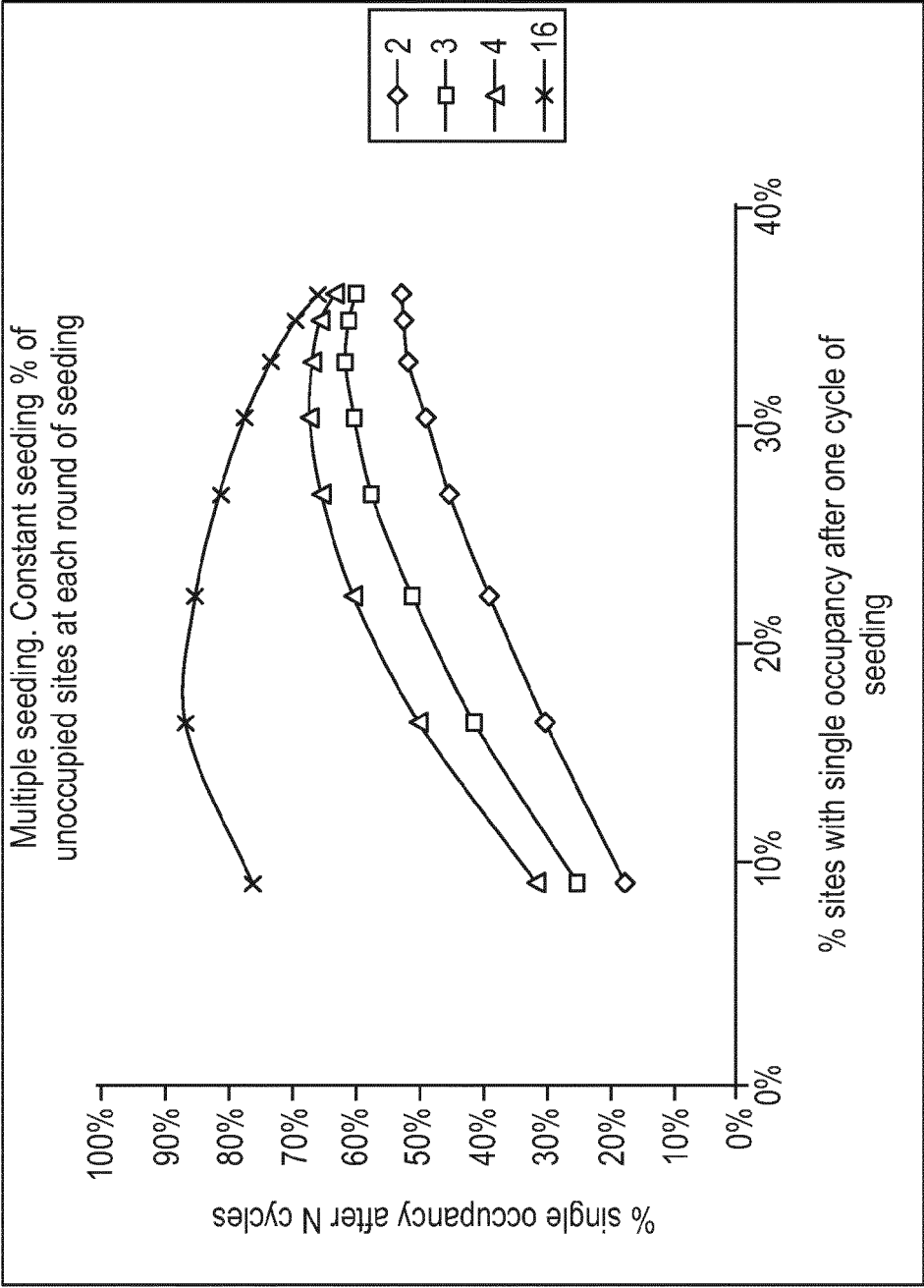


Fig. 17

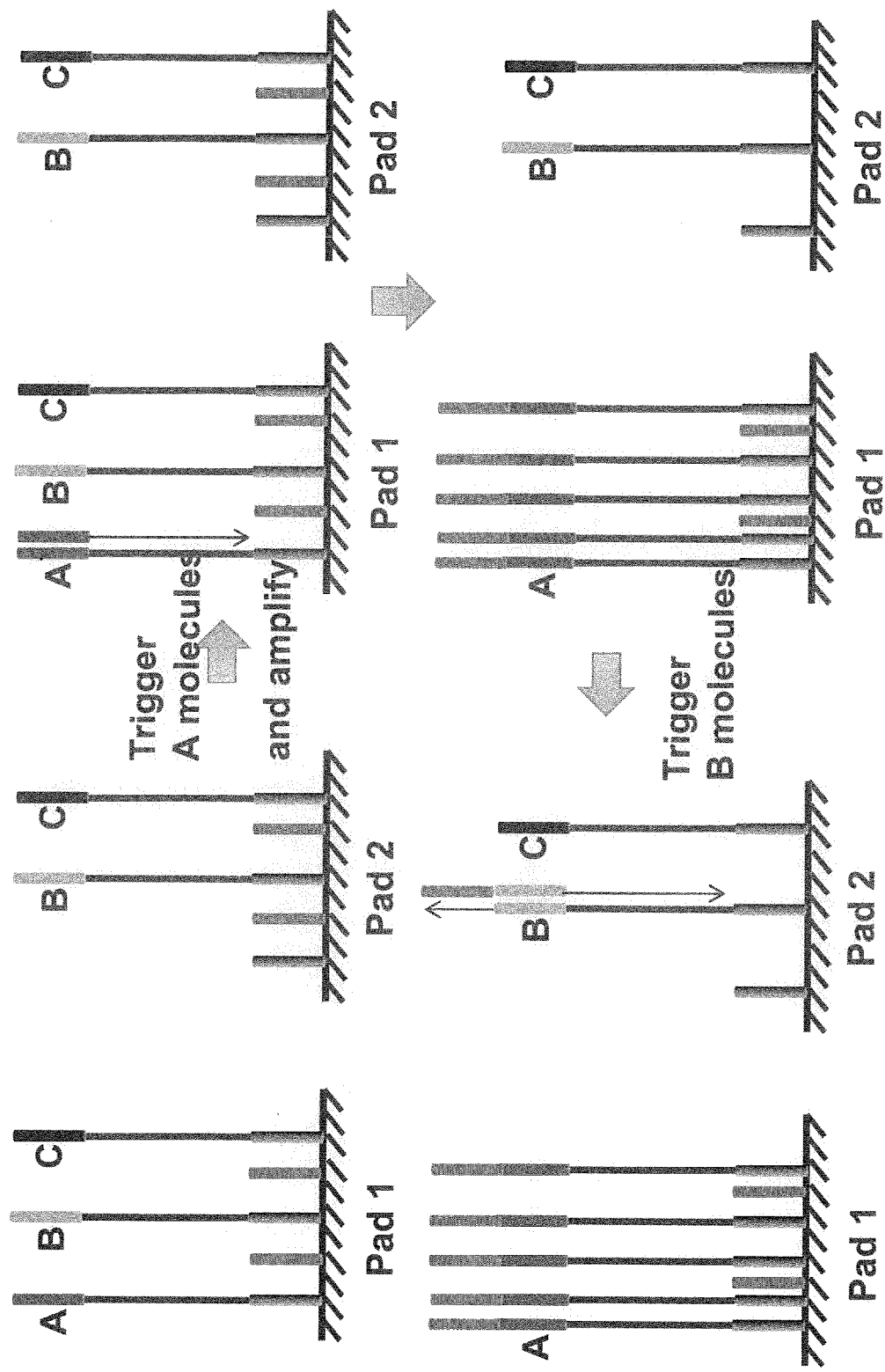
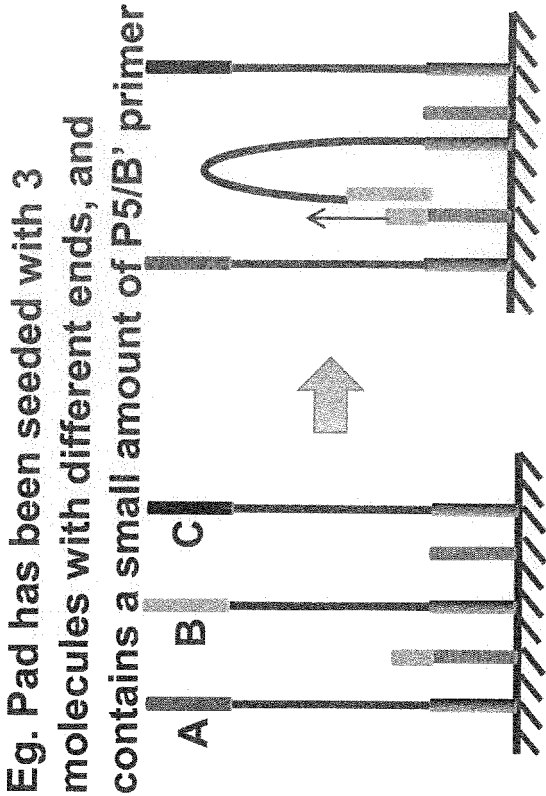


FIG. 18



At some point during incubation
molecule B hybridizes to P5/B', which
is extended and starts amplification of that pad

- ▶ Other pads would contain eg. P5/A' or P5/C' to enable amplification of those molecules

FIG. 19

Cleavable hairpin approach

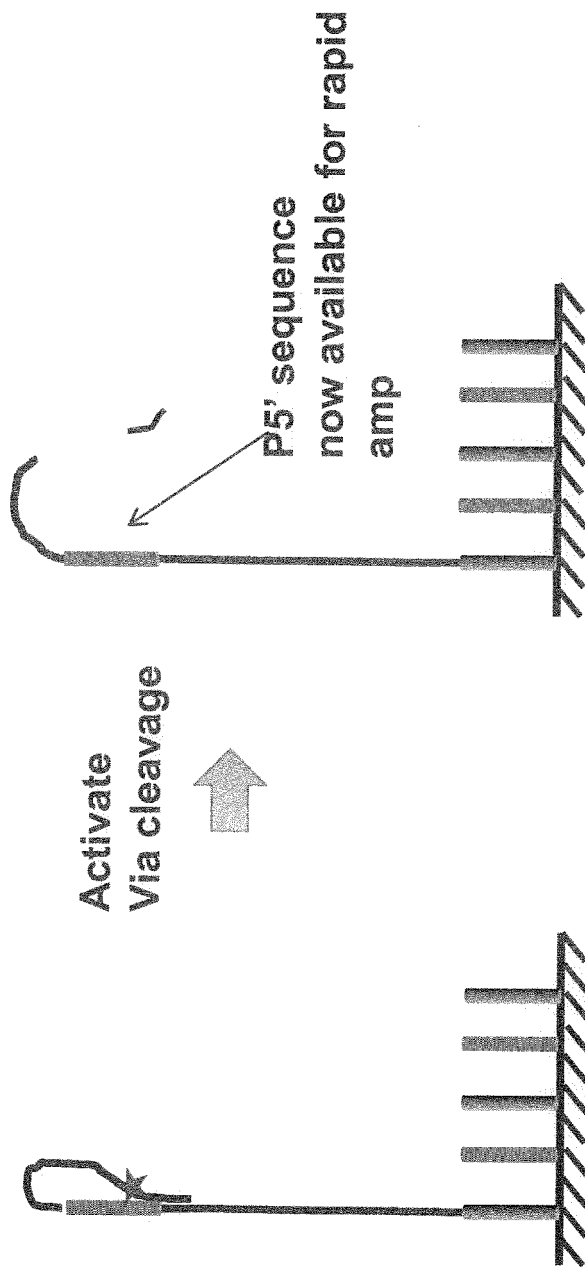
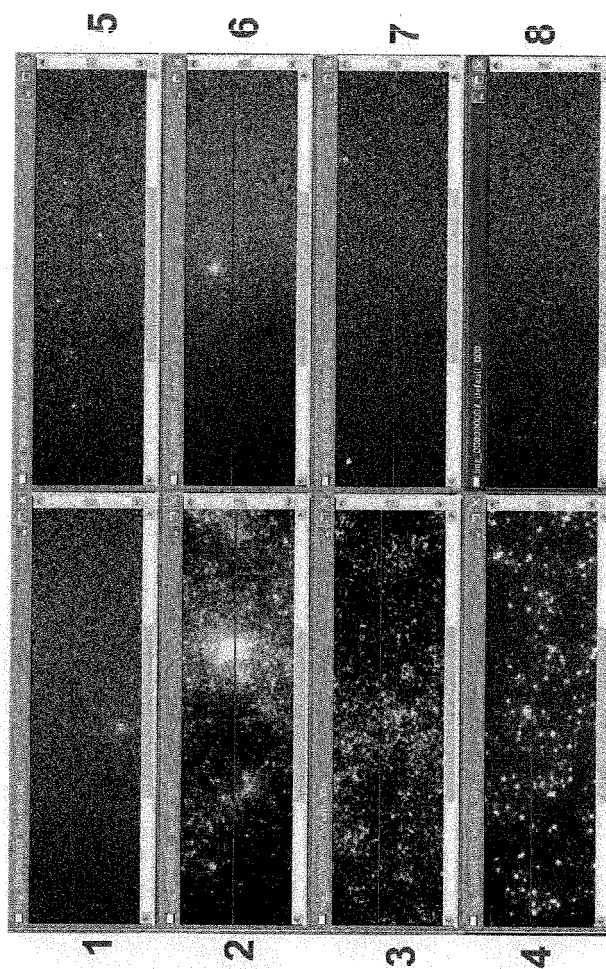


FIG. 20

Activation of amplification by P5/SBS3 in KEA



- ▲ Random P5/P7 surface flowcell seeded with templates ending in SBS3 but lacking a P5 sequence in lanes 1 to 5, no template controls in lanes 6 to 8
- ▲ Subsequent KEA used v2 KEA with or without P5/SBS3 oligo show formation of clusters when oligo included (lanes 2 to 4), but not without oligo (lane 1) or without template (lanes 6 to 8)

FIG. 21